Considerations and Recommendations to Standard Testing with Daphnia magna

Cupi, Denisa; Baun, Anders

Publication date:
2013

DTU Environment
Department of Environmental Engineering

Technical University of Denmark

TU066

Considerations and Recommendations to Standard Testing with *Daphnia magna*

Denisa Cupi and Anders Baun
denc@env.dtu.dk

Introduction and Aim

OECD standard tests are useful tools for testing and ranking chemicals. As part of FP7 Project MARINA (Managing Risks of Nanomaterials), this research aimed at developing and harmonizing specific reference methods for testing engineered nanoparticles accounting for their novel characteristics.

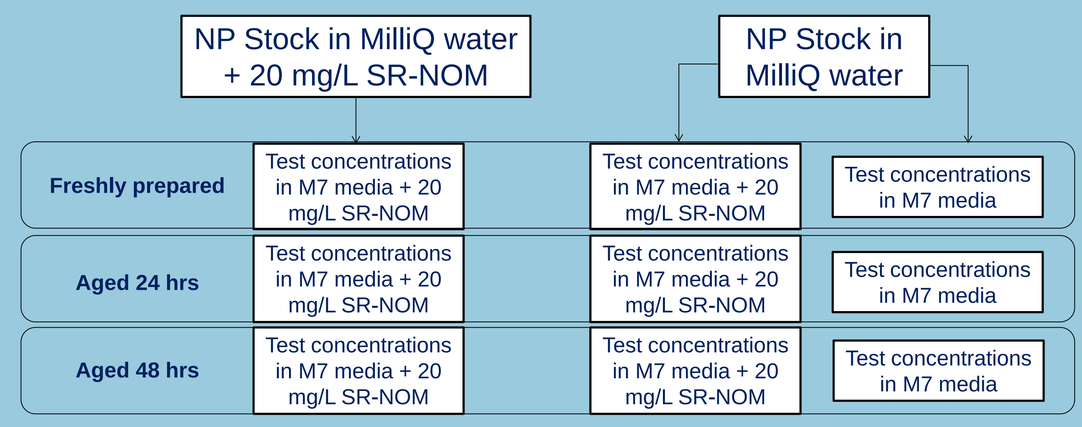
OECD reference nanoparticles TiO_2 (NM-104), TiO_2 (NM-105), Ag (NM-300K), CeO_2 (NM-212), ZnO (NM-110), ZnO (NM-111), SiO_2 (NM-200) were employed to test acute toxicity on freshwater crustacean *Daphnia magna* using OECD 202 guideline.

Due to challenges encountered during this testing and taking into consideration environmental relevance, we used three NPs TiO_2 (NM-104), Ag (NM-300K), ZnO (NM-110), to investigate:

-The procedure for addition of SR-NOM
- If SR-NOM can stabilize stock suspensions
- The effect that SR-NOM has on toxicity and size distribution of NPs

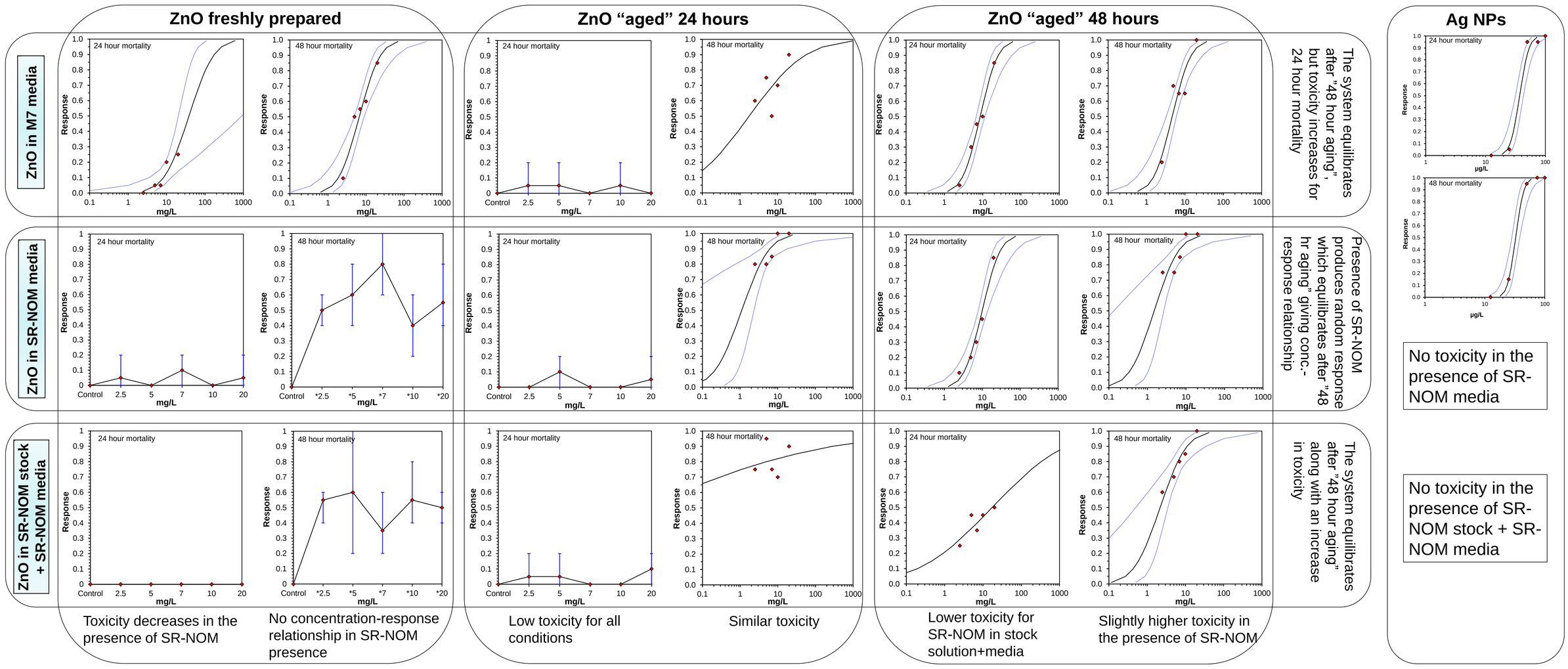
-The effect that "aging" (for 24 and 48 hours) of test concentrations has on agglomeration and toxicity of NPs.

Experimental Setup and Methods



- Samples were sonicated for 20 minutes using Digital sonifier Model 250 by Branson
- NPs size and charge were measured by DLS (Zeta Sizer Nano)
- NTA (Nanosight) measurements were attempted
- Juvenile daphnids were exposed to different concentrations according to OECD 202 guideline
- ToxCalc was used to generate concentration-response curves and graphs

Results



DLS + NTA

Stock solutions and test concentrations measured before and after aging		ZnO (NM-110) Size range (nm)	ZnO (NM-110) PDI range	ZnO (NM-110) Charge range (mV)	Ag (NM-300K) Size range (nm)	Ag (NM-300K) PDI range	Ag (NM-300K) Charge range (mV)
0 hours	Stock in SR-NOM H_2O	133	0.18	-35.6	29	0.43	-17.1
	Test concentrations in SR-NOM stock + SR-NOM media	133-198	0.18-0.31	-18.0 to -19.3	385-896	0.85-1.00	-5.7 to -6.8
	Stock in H_2O	*132*	*0.09*	*33.6*	*63*	*0.25*	*-19.0*
	Test concentrations in M7 media	*380-638*	*0.24-0.54*	*-2.1 to -6.4*	*461-821*	*0.88-1.00*	*-6.9 to -9.73*
	Test concentrations in SR-NOM media	*136-138*	*0.16-0.30*	*-17.5 to -18.0*	*258-616*	*0.73-0.90*	*-6.2 to -7.2*
24 hours	Stock in SR-NOM H_2O	135	0.15	-25.5	39	0.19	-32.4
	Test concentrations in SR-NOM stock + SR-NOM media	133-188	0.20-0.32	-14.6 to -17.4	657-667	0.90-1.00	-7.2 t -8.1
	Stock in H_2O	*145*	*0.24*	*27.8*	*44*	*0.23*	*-22.4*
	Test concentrations in M7 media	*1644-3092*	*1.00*	*-2.9 to -5.9*	*347-1032*	*0.77-1.00*	*-9.7 to -10.1*
	Test concentrations in SR-NOM media	*140-145*	*0.20-0.35*	*-14.4 to -17.1*	*555-738*	*0.93-1.00*	*-6.0 to -7.7*
48 hours	Stock in SR-NOM H_2O	133	0.14	-21.9	74	0.17	-36.2
	Test concentrations in SR-NOM stock + SR-NOM media	144-162	0.26-0.40	-14.4 to -16.0	515-987	1.00	-6.9 to -9.0
	Stock in H_2O	*240*	*0.56*	*23.3*	*52*	*0.24*	*-20.5*
	Test concentrations in M7 media	*1303-3506*	*1.00*	*-3.6 to -8.1*	*390-1316*	*0.86-1.00*	*-7.3 to -14-4*
	Test concentrations in SR-NOM media	*177-208*	*0.32-0.44*	*-12.9 to -14.7*	*296-1789*	*0.72- 1.00*	*-7.8 to 10.3*

ZnO and Ag NP stock sol. were more stable over time than test conc. in media, whereas **TiO_2 NPs** stock sol. greatly agglomerated over time. For all NPs, use of M7 media lead to greater size particles than SR-NOM M7.

Conclusions

The toxicity of nanoparticles to *Daphnia magna* ranked in the following order Ag > ZnO (NM-110) > ZnO (NM-111) > CeO_2 > TiO_2 > SiO_2.

Addition of 20 mg/L SR-NOM in the stock and the SR-NOM media:

- Lead to an imbalanced system at "0 hr and 24 hr aging" of ZnO NPs.
- Decreased toxicity of Ag NPs in all exposure conditions and therefore is recommended to not be used while testing these nanoparticles for toxicity.
- Aided in the stability of the test concentrations of both nanoparticles leading to a smaller size and less sedimentation compared to those in M7 media.

"Aging" of test concentrations caused:

- More stable system and an increase in toxicity for ZnO NPs at "48 hr aging".
- No significant difference in toxicity for Ag NPs as they seem to exhibit most effect within the first 24 hours of exposure.
- Increase in size and sedimentation for ZnO, Ag and more markedly for TiO_2 NPs.

Acknowledgment: This research was funded by FP7 Project MARINA (Managing Risks of Nanomaterials)